Impact of introducing alien storage proteins on the bread-making quality and agronomic performance of spring wheat

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The relatively narrow genetic base of spring wheat grown in western Canada puts the crop at risk from attack by pests which reduce yield and marketability. Interspecific hybridization is used to introduce new genetic sources of resistance to wheat pests. This study was conducted in order to evaluate the effectiveness of endosperm protein fingerprinting in selecting backcross-derived (BC) lines from the cross Park*3/ T. timopheevi. SDS-PAGE and A-PAGE were used to identify the high molecular weight (HMW) glutenin subunit and gliadin constitution, respectively, of the BC lines. Although no exotic HMW glutenin subunits were present in the BC lines, a high frequency lacked the 'A' genome subunit. This 'null' condition was deleterious to bread-making quality. The BC lines with the null condition were characterized by the transfer of novel omega and gamma gliadin proteins from T. timopheevi and kernel texture normally associated with tetraploid wheat. Despite the level of back-crossing, a large majority of the lines were inferior to the recurrent parent for agronomic performance. Delayed heading, resulting from the putative transfer of a major maturity gene from T. timopheevi, was associated with low grain yield in 1991 but higher yield in 1992. Lines with improved baking quality tended to be low-yielding. Based on this study, protein fingerprinting can be used to select interspecific lines with desirable end-use quality.

INTRODUCTION

Approximately 40% of wheat endosperm protein consists of glutenins. Certain high molecular weight (HMW) glutenin subunits in common wheat are associated with high breadmaking quality (Payne et al., 1987) Canadian Western Red Spring (CWRS) wheat cultivars carry the alleles associated with the highest levels of bread-making quality (Luckow et al., 1989) as do most breeders' experimental lines currently in cooperative registration trials. Government legislation dictating end- use quality levels and kernel visual distinguishability has resulted in CWRS wheat breeding programs with a relatively narrow germplasm base. Consequently, HMW glutenin subunit identification will provide little or no information to wheat breeders selecting in populations derived from adapted x adapted crosses. In the latter situation, protein quality being similar, protein quantity is of greater importance. The introduction of disease resistance genes from related species or exotic hexaploid wheats has been a slow, and in many cases, unsuccessful effort due to undesirable quality traits in the disease resistant segregants (Knott, 1989). With the current emphasis on reduced pesticide use, for environmental and economic reasons, breeding for enhanced resistance to pests is increasing in importance.

The objectives of this study were: (1) to identify the High Molecular Weight (HMW) glutenin subunits present in selected interspecific backcross-derived lines of spring wheat (Triticum aestivum L.), (2) to determine the effectiveness of selecting specific HMW glutenin subunits in predicting over-all end-use quality and (3) to determine the relationship between bread-making quality and agronomic performance in this material.

MATERIALS AND METHODS

Field Experiments

T. timopheevi accession TT12 (PI290518) was used as a donor parent in order to develop BC2F4 lines with the recurrent parent, cv Park. TT12 was identified as resistant to S.

nodorum by Dr. Hong Ma as part of his M.Sc. project (Dept. of Crop Science & Plant Ecology, University of Saskatchewan, 1988). The BC-derived lines and their parents were grown in unreplicated single rows in the field in 1990. Lines with sufficient seed were evaluated in 1991. Fifty-nine (59) lines of Park*3/TT12 were grown in 2 and 3-replicate RCBD experiments near Saskatoon in 1991 and 1992, respectively. The cultivar Park was used as a control.

Each plot consisted of four rows spaced 30 cm apart. The row length was 2.4 m in 1991 and 3.6 m in 1992. The seeding rate was ca. 200 seeds/m2 (1991) and 250 seeds/m2 (1992). The experiments were seeded on 22 May, 1991 and 19 May, 1992.

The following agronomic data were collected on two plots/line in both years:

1. Days to 50% spike emergence (DSE).
2. Days to 95% physiological maturity (DPM).
3. Plant height.
4. Grain yield (3 reps in 1992).
5. Test weigh.
6. Kernel weight.
7. Lodging (Belgian system) in 1992 only.

The following quality characteristics were measured on each plot:

8. Flour yield and moisture (AACC method 44-15A).
9. SDS sedimentation (modified Axford et al. 1978).
10. Flour protein (Udy, AACC method 46-14A).
11. Mixogram (AACC method 54-40).
12. Bake test (Remix method, AACC method 10-10A).
13. Kernel hardness (1992), (Brabender SM1 tester method).

HMW glutenin and gliadin characterization

Glutenin was extracted using methodology described by Graveland et al. (1980) and Khan et al. (1989). Single dimensional SDS-PAGE was carried out on 17.3% (w/v) separating and 3% (w/v) stacking gels, respectively, according to modified methods of Payne et al. Gels were stained with Coomasie R250.

Each of the lines included in the 1991 field experiment was fingerprinted for HMW glutenin subunits. Seed grown in 1990 was used for electrophoresis assays. Five separately hand-harvested plants per genotype were assayed for HMW glutenin subunits. Four seeds were bulked from each plant for SDS-PAGE assays. Acid-PAGE (Ng et al., 1988) was conducted on 1990 and 1991 seed.

RESULTS AND DISCUSSION

SDS-PAGE analysis of Park*3/TT12 lines indicated that there were two groups of lines. One group had the HMW glutenin constitution of the recurrent parent (1, 7+9, 5+10) while the second group of lines lacked 'A' genome subunits (i.e. were null lines). The latter group of lines was, however, homozygous at the 'B' and 'D' genome HMW loci. The BC lines traced back to four original F1 hybrids. Lines derived from two of the four hybrids exhibited either sub-unit 1 or were nulls (Families 2 and 4). Lines derived from the other two F1 hybrids all carried subunit 1 (Families 1 and 3).

Acid PAGE and SDS PAGE analysis revealed that a majority (75%) of null lines also carried novel gliadin proteins transferred from T. timopheevi. Omega gliadins present in Park were absent in BC lines exhibiting the T. timopheevi bands, suggesting that inactive TT12 omega gliadins were transferred. Gamma and omega gliadin genes are tightly linked on the short arm of chromosome 1AS. Most of the lines which had the null type composition also had gliadin transfers. It is likely that chromosome 1A of Park which codes for HMW glutenin subunit 1 and omega gliadins, has been fully or partially replaced in the backcross program by segments of T. timopheevi 1A chromosome.

Very large differences in grain yield were observed among genotypes within the population derived from Park*3/TT12 in both 1991 and 1992. In large part, the agronomic differences resulted from an apparent vernalization requirement, or late maturity per se, for approximately half of the genotypes (Table 2). An approximate two week difference in heading time was observed among genotypes in each of three years (1990, 1991 and 1992). It would appear that the apparent vernalization requirement or a major gene for late maturity from TT12 was transferred in this interspecific cross. The late-maturing BC group had a maturity requirement that coincided with that of TT12 while the early maturing group coincided with Park (Table 1). This bimodal distribution suggests that a single gene system is involved, favouring a hypothesis of unmet vernalization requirement in the late maturing genotypes.

Due to very different growing conditions in 1991 and 1992, the late maturing group of lines either yielded significantly less or significantly more than the recurrent parent. Due to this genotype x year interaction, the data was not averaged over years (Tables 2 to 5).

Genotypes which were late-heading tended to be low-yielding (r=0.86**) in 1991 but not so in 1992 (r=-0.03) (Table 2). Delayed heading resulted in lower test weights (r=0.69**, 0.79**) and kernel weights (r=0.51**, 0.43**) in both years. In 1991, the majority of BC-derived lines yielded less than the cultivar Park, however, a number of genotypes were equal to or slightly higher yielding than the recurrent parent (Table 2). In 1992, the majority of BC genotypes yielded equal to or better than Park. In 1991 late season drought curtailed grain-filling in the late maturing lines, reducing their yield by up to 50% relative to Park. In 1992, however, cool moist conditions extended the grain-filling period, putting later-maturing material at an advantage over Park (Table 2). As a result of curtailed grain-filling, late-maturing BC genotypes had a higher protein content (r=0.55**) in 1991 (Table 4). In 1992 however, delayed maturity was associated with lower protein levels (r=-0.74**). Late maturing genotypes were taller (r=0.58** (1991), 0.84** (1992)) and tended to lodge more severely (r=0.85**) than earlier maturing genotypes.

Most of the null genotypes had very hard kernels relative to lines carrying HMW 1. In 1992, the mean kernel hardness of null lines (expressed in grinding time) was 15.5 seconds compared to 18.6 for the HMW 1 group. For Park, the grinding time was 24 seconds (Table 1). A lower value is associated with a harder kernel texture. The grinding time of TT12 was 13.6 seconds.

Predictive quality tests (SDS Sedimentation, Mixograph) indicated that a majority of the Park*3/TT12 lines had weaker gluten characteristics than Park, but a higher protein content (Tables 4 & 5). The lines which had a higher protein content than Park were lower yielding. Late maturity was associated with lower sedimentation volumes (r=-0.5** to -0.8**) in both years, indicating that the late-maturing group had a poorer protein quality.

In 1992 the later maturing BC genotypes were affected by an early fall frost. Consequently, protein quantity and quality effects on end-use quality were confounded by maturity ffects. In 1991, in the absence of frost damage, the null condition resulted in a 7%

reduction in loaf volume relative to the HMW 1 condition (892 vs 961 cc). In 1992, a similar reduction was observed (715 vs 769 cc). Due to the small sample sizes these differences were not statistically significant.

Late maturity was associated with kernel hardness (r=-0.86**) indicating that three traits have been transferred, as a block, from TT12: hard kernels, vernalization requirement, novel proteins. The above suggests that a fairly large segment of chromosome has likely been introgressed from T. timopheevi. As a result of this linkage it is difficult to determine the effects of protein transfers on end-use quality.

Principal Component Regression (PCR) models were developed which included data from densitometric measurements of SDS-PAGE gels run on 1991 flour samples, protein content, and kernel hardness. This statistical analysis was conducted in order to separate out the quantitative effects of HMW glutenin subunits, gliadins and other low molecular weight proteins on end-use quality (Battacharjee, 1993).

Depending on the year, between 54 and 61% of the variation in SDS sedimentation volume, 13% of the variation in mixograph area and 8 to 33% of the variation in loaf volume was explained by the three factors (protein composition, protein content, kernel texture). The HMW gutenins had a positive effect on quality while the introduced gliadin proteins had a negative effect. Short grinding time (i.e. harder endosperm) had a negative effect on quality. The effect of protein content on quality was inconsistent.

CONCLUSIONS

SDS-PAGE was used successfully to identify the HMW glutenin subunit constitution of interspecific BC-derived lines. Although no exotic HMW glutenin subunits were recovered in the BC genotypes, a large proportion of Park*3/TT12 genotypes lacked the 'A' genome HMW subunit. This 'null' condition is known to be deleterious to bread-making quality. The genotypes with the null condition were, however, characterized by the transfer of apparently novel gliadin proteins from T. timopheevi as well as kernel texture (hardness) normally associated with tetraploid wheats. The genotypes used in this study were the result of two back-crosses to the cultivar Park. Despite the back-crossing, a majority of genotypes were inferior to the recurrent parent for agronomic performance. Delayed heading, resulting from the putative transfer of a major gene for vernalization requirement from T. timopheevi, was associated with low grain yields in 1991 but higher yields in 1992. Based on this study, SDS-PAGE and Acid-PAGE can be used in early generations to cull genotypes with undesirable end-use quality in interspecific wheat populations.

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Table 1. Agronomic and quality traits of Park and TT12 grown at Saskatoon in 1992.

	Grain yield z (kg/ha)	DSE (d)	DPM (d)	Plant height (cm)	Lodging score (0.2-9)
Park	3959±40	55.0±0.0	97.0±0.0	97.0±2.0	2.0±0.4
TT12	3290±93	61.5±4.5	108.0±7.0	116.5±0.5	2.1±0.3

	Test weight (kg/hL)	Grinding time (sec)	Flour protein z (%)
Park	79.9±0.3	24.0±0.0	15.4±0.1
TT12	73.3±0.2	13.6±0.3	14.2±0.1

z Means and standard errors were calculated on three replications. For the remaining traits, n=2.
DSE = days to 50% spike emergence, DPM = days to 95% physiological maturity.

Table 2. Agronomic performance of Park*3/TT12 families and sub-families differing in glutenin and gliadin composition.

Family		N	Grain yield (kg/ha) 1991	Grain yield (kg/ha) 1992	DSE (d) 1991	DSE (d) 1992
Park	(1-G1)		3596±145	3560±106	49.0±1.0	49.5±0.5
Family 1	(1-G1)	11	3547±56	3693±54	48.7±0.2	49.4±02
Family 3	(1-G1)	4	2544±243	3081±46	51.2±1.0	51.5±0.2
Family 2	(1-G1)	6	2089±300	3306±84	58.5±3.9	55.8±2.5
Family 2	(1+G1)	4	2454±435	3355±93	58.8±3.9	58.4±3.4
Family 2	(N+G1)	9	1446±205	3011±88	65.4±3.5	63.6±2.4
Family 2	(N-G1)	1	1867	2990	56.5	59.0
Family 2	(Average)	20	1894±174	3167±60	61.2±2.1	60.0±1.6
Family 4	(1-G1)	2	2294±125	3795±63	55.2±0.7	53.7±0.7
Family 4	(1+G1)	6	1987±85	3706±112	63.7±0.6	61.9±0.5
Family 4	(N+G1)	12	1880±99	3899±68	65.6±0.5	63.3±0.2
Family 4	(N-G1)	4	2190±142	4041±197	62.1±2.6	61.7±2.4
Family 4	(Average)	24	1993±64	3866±57	63.7±0.8	61.9±0.7

DSE = days to 50% spike emergence.

Table 3. Agronomic and quality performance of Park*3/TT12 families and sub-families differing in glutenin and gliadin composition.

Family		N	Plant height (cm) 1991	Plant height (cm) 1992	Grinding time (sec) 1991	Grinding time (sec) 1992
Park	(1-G1)		99.0±1.0	87.5±1.5	20.5±0.5	24.5±0.5
Family 1	(1-G1)	11	99.7±0.5	88.9±0.5	19.9±0.2	22.4±0.4
Family 3	(1-G1)	4	89.6±1.7	84.6±1.3	18.1±0.5	19.7±0.8
Family 2	(1-G1)	6	105.0±1.4	100.9±2.7	20.4±1.0	19.6±1.6
Family 2	(1+G1)	4	104.7±0.5	98.2±4.0	16.4±0.2	16.4±1.2
Family 2	(N+G1)	9	112.1±1.5	112.8±1.0	15.6±0.1	14.8±0.3
Family 2	(N-G1)	1	106.5	101.0	17.0	15.0
Family 2	(Average)	20	107.8±1.1	105.7±1.9	17.4±0.6	16.5±0.7
Family 4	(1-G1)	2	106.0±3.5	101.2±1.2	19.5±0.5	18.0±0.5
Family 4	(1+G1)	6	108.6±1.4	108.6±1.5	16.5±0.4	15.6±0.3
Family 4	(N+G1)	12	108.0±0.8	107.5±0.8	16.6±0.2	15.4±0.2
Family 4	(N-G1)	4	108.0±0.7	109.0±0.3	19.4±0.1	17.5±0.4
Family 4	(Average)	24	108.3±0.6	107.5±0.7	17.3±0.3	16.0±0.2

1-G1 = HMW1 without TT12 gliadin transfer; 1+G1 = HMW1 with TT12 gliadin transfer; N-G1 = null condition without TT12 gliadin transfer; N+G1 = null condition with TT12 gliadin transfer.

Table 4. Quality performance of Park*3/TT12 families and sub-families differing in glutenin and gliadin composition.

Family		N	Flour protein (%) 1991	Flour protein (%) 1992	SDS sedimentation (cc) 1991	SDS sedimentation (cc) 1992
Park	(1-G1)		16.2±0.6	15.6±0.1	80.7±2.7	77.0±3.0
Family 1	(1-G1)	11	16.4±0.2	15.9±0.1	81.7±0.9	76.2±0.9
Family 3	(1-G1)	4	16.8±0.4	16.3±0.2	68.8±2.6	68.7±3.7
Family 2	(1-G1)	6	17.1±0.3	14.8±0.3	83.7±1.3	72.0±4.8
Family 2	(1+G1)	4	17.1±0.1	15.2±0.6	74.7±2.1	62.4±6.8
Family 2	(N+G1)	9	17.4±0.2	14.1±0.2	69.8±1.2	45.5±2.0
Family 2	(N-G1)	1	17.1	15.0	85.2	59.0
Family 2	(Average)	20	17.2±0.1	14.5±0.2	76.4±1.6	57.5±3.3
Family 4	(1-G1)	2	16.8±0.3	15.1±0.0	79.1±3.6	63.4±2.6
Family 4	(1+G1)	6	17.4±0.4	15.1±0.2	65.4±2.6	56.6±2.0
Family 4	(N+G1)	12	17.3±0.1	15.0±0.1	64.4±1.6	53.6±2.0
Family 4	(N-G1)	4	17.4±0.4	15.0±0.2	78.5±2.0	64.9±3.8
Family 4	(Average)	24	17.3±0.1	15.0±0.1	68.2±1.6	57.0±1.5

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Table 5. Quality performance of Park*3/TT12 families and sub-families differing in glutenin and gliadin composition.

Family		N	Mixograph development time (min) 1991	Mixograph development time (min) 1992	Loaf volume (cc) 1991	Loaf volume (cc) 1992
Park	(1-G1)		3.2±0.2	3.0±0.3	920±40	840±10
Family 1	(1-G1)	11	3.1±0.1	2.7±0.1	897±18	854±6
Family 3	(1-G1)	4	2.8±0.1	2.8±0.2	898±16	904±13
Family 2	(1-G1)	6	3.8±0.1	4.4±0.1	925±28	780±22
Family 2	(1+G1)	4	2.9±0.2	3.6±0.4	929±36	779±42
Family 2	(N+G1)	9	2.9±0.1	3.6±0.1	869±21	573±30
Family 2	(N-G1)	1	3.8	4.2	822	665
Family 2	(Average)	20	3.3±0.1	3.9±0.1	898±16	681±28
Family 4	(1-G1)	2	3.4±0.3	3.3±0.1	894±14	867±62
Family 4	(1+G1)	6	2.6±0.1	2.8±0.1	831±28	800±19
Family 4	(N+G1)	12	2.6±0.1	2.8±0.1	844±12	760±12
Family 4	(N-G1)	4	3.2±0.2	3.4±0.1	883±52	800±14
Family 4	(Average)	24	2.7±0.1	3.0±0.1	852±12	786±11